Long-Term Recovery of Microbial Communities in the Boreal Bryosphere Following Fire Disturbance

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Long-term recovery of microbial communities in the boreal bryosphere following fire disturbance

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ABSTRACT

Our study used a ~360-year fire chronosquence in northern Sweden to investigate post-fire microbial community dynamics in the boreal bryosphere (the living and dead parts of the feather moss layer on the forest floor, along with the associated biota). We anticipated systematic changes in microbial community structure and growth strategy with increasing time since fire (TSF) and used amplicon pyrosequencing to establish microbial community structure. We also recorded edaphic factors (relating to pH, C and N accumulation) and the physical characteristics of the feather moss layer. The molecular analyses revealed an unexpectedly diverse microbial community. The structure of the community could be largely explained by just two factors, TSF and pH, although the importance of TSF diminished as the forest recovered from disturbance. The microbial communities on the youngest site (TSF = 14 yrs) were clearly different from older locations (> 100 yrs), suggesting relatively rapid post-fire recovery. A shift towards Proteobacterial taxa on older sites, coupled with a decline in the relative abundance of Acidobacteria, suggested an increase in resource availability with TSF. Saprotrophs dominated the fungal community. Mycorrhizal fungi appeared to decline in abundance with TSF, possibly due to changing N status. Our study provided evidence for the decadal-scale legacy of burning, with implications for boreal forests that are expected to experience more frequent burns over the course of the next century.

Key-words: boreal forest, climate change, microbial community structure, feather mosses, nutrient cycling, post-fire succession

INTRODUCTION

Bryophytes are important components in many ecosystems, including bogs, boreal forests and the biological soil crusts of arid regions. Microbes living on moss tissue can have great ecological significance in these habitats [1-4]. The boreal bryosphere (i.e., the living and dead parts of the feather moss layer on the boreal forest floor, along with the associated biota) is particularly significant [5]. It is spatially extensive, accounts for a significant proportion of boreal forest biomass and plays a critical role in biogeochemical cycling [6]. The ecological function of microbes dwelling in the boreal bryosphere is disrupted by fire, a disturbance which may increase in severity and/or frequency with climate change [7]. However, whilst there has been extensive research into the effects of burning on communities of plants and animals, relatively little work has been carried out on the long-term (decades – centuries) fate of microbial communities following fire disturbance. We therefore set out to infer long-term changes in the bacterial and fungal communities of the boreal bryosphere following fire disturbance. We also aimed to relate these changes to disturbance-related shifts in environmental (primarily edaphic) conditions.

Upland moss communities of the boreal forest are dominated by two pleurocarpous moss species: *Pleurozium schreberi* (Brid.) Mitt., *Hylocomium splendens* (Hedw.) Schimp. and the acrocarpous moss *Polytrichum commune* (Hedw.) *Hylocomium* and *Pleurozium* can account for 30 to 95% of average cover of the boreal forest floor, yield a net primary productivity (NPP) of 240 – 440 kg ha^{-1} yr^{-1} and a total biomass of up to 2000 kg C ha^{-1} [8,9]. This photosynthetic capacity is reflected in an ecosystem productivity that rivals and at times (early to mid-succession) surpasses that of the overstory [10]. Further, the moss bottom layer serves a variety of functions in the boreal forest, acting to detoxify water, regulate nutrient uptake and minimize nutrient loss [5].

To a large extent, the microbes of the moss layer underpin the cycling of carbon (C) and nitrogen (N) in boreal forests. DeLuca et al. [11] estimated that Cyanobacteria living in the bryosphere are responsible for the fixation of 1.5 – 2.0 kg N ha^{-1} yr^{-1} in mid- to late-successional boreal forests. Cyanobacterial activity is therefore a major determinant of boreal forest productivity because these ecosystems are N limited [12]. Heterotrophic organisms which feed on or interact with Cyanobacteria

within the bryosphere are likely to play a key role in the turnover and cycling of fixed N at an ecosystem level [5].

Microbially-mediated biogeochemical processes are disrupted by fires that affect the moss and underlying humus layer. Aside from obvious losses of microbial biomass [13], previous studies have shown that burning releases a pulse of mineralised N into the soil, resulting in higher N turnover rates and availability [14]. Burning also reduces soil organic carbon, promotes the formation of chemically recalcitrant charcoal and increases pH, all of which have long-term impacts on ecosystem function.

Temporal changes in microbial community structure following disturbance have been studied before and there is a small body of work on the post-fire recovery of *soil* microbial communities [SMCs: see, e.g., 13,15,16-18]. In addition, microorganisms living in boreal forests, particularly soil fungi, have been the subject of previous studies [see, e.g. 19,20-23]. However, whilst a number of researchers have investigated comparatively short-term microbial succession, few have concentrated on long-term (decades-centuries) changes in community composition and structure [24]. Furthermore, with a few notable exceptions [25,26], microbes dwelling in the bryosphere have received little attention. This is, to our knowledge, the first study to encompass both the bacterial and fungal communities of the boreal bryosphere.

Previous research has demonstrated that SMCs are heavily influenced by edaphic factors [27-29]. It is reasonable to assume that microbes in the bryosphere respond to similar factors, as bryophytes form a continuous interface between the soil organic layer and the forest understory. In a spatially extensive study, Fierer and Jackson [30] found that the abundance of major bacterial phyla varied systematically according to resource availability. In soils where resources were plentiful, communities were dominated by bacteria with copiotrophic (r-selected) growth strategies. These bacteria preferentially consume labile C, have high nutritional requirements and high growth rates. Conversely, where resources were limited, oligotrophic (K-selected) soil bacteria were more prevalent. Studies of soil fungi have also highlighted differences in life history strategy related to edaphic factors. For example, Lindahl et al. [31] found that newly shed litter on the floor of a boreal forest was dominated by saprotrophic fungi, which were more efficient at utilising fresh plant tissue. More decomposed

substrates were characterised by a) 'late' colonizing saprotrophs capable of metabolising recalcitrant substrates and b) mycorrhizal (MR) fungi. Given the significance of environmental factors to microbial community structure, it is reasonable to posit that microbial communities in and around the soil environment will respond to the progressive changes in edaphic factors that accompany post-fire succession.

The bryosphere is an intermediate layer between below- and above-ground components of the boreal forest [32] and, as such, it is influenced by both the underlying soil and the forest canopy. Even though the feather mosses that make up much of the bryosphere have non-vascular rhizoids rather than roots, previous research has demonstrated that changes in nutrient availability during succession may be reflected in the chemistry of moss tissue [see, e.g., 33] and direct uptake of C and N from the soil has been described in feather moss species such as *P. schreberi* [34]. Higher N turnover during early succession results in higher N deposition and availability after a fire [35], which may also influence microbial community composition in the bryosphere. We therefore anticipated that microbial community composition and structure in the bryosphere would vary systematically with time since fire (TSF). We expected r-selected bacteria to dominate shortly after disturbance, as these taxa can proliferate rapidly when resources are plentiful (e.g. mineralised nutrients released by burning and labile organic matter in the form of dead roots and microbial cells). K-selected bacteria should be dominant on older sites where diverse communities have established, nutrients have been leached or tied-up in chemically-recalcitrant organic compounds and nutrient cycles are less 'leaky'. Similarly, we anticipated saprotrophic fungi would dominate on 'young', regenerating sites. On older sites, where trees and ericaceous shrubs have become established, we anticipated a higher proportion of mycorrhizal fungi [36].

METHODS

We investigated long-term changes in microbial community structure using space-for-time substitution, i.e. by comparing sites that only varied in terms of TSF (commonly referred to as a fire chronosequence). This approach has well known limitations [37]. For example, in studies utilising fire chronosequences there are, by definition, no control sites (unburnt locations of a similar age).

Furthermore, the patchy nature of fire disturbance makes it difficult (frequently impossible) to identify replicate sites. Despite these limitations, the use of chronosequence studies has gained wide acceptance in ecology [38-40], including microbial ecology [16,41-43]. Indeed, in many circumstances it may be the only way of gaining a long-term perspective on ecological processes [44]. Most commentators agree that the most important consideration in such studies is good age control [37].

STUDY SITE

The study was conducted in the boreal forest of northern Sweden, utilising five sites that formed a 363-year fire chronosequence (Table 1). The characteristics of the sites have been described in detail elsewhere [45-47]. The natural fire return period for this habitat has been estimated to be ~60 years [48]. Vegetation succession following fires is relatively slow: locations < ~80 years since burning are considered to be in the early stages of succession; those ~100 – 200 years are mid-succession, and sites >200 years are in the later stages of succession [46]. The sites were chosen to a) minimise differences in state factors, other than TSF and b) to encompass a broad age range, with an even temporal spacing of sites at early, mid and late stages of forest succession. The soils on the sites were podzolic (Typic or Entic Haplocryods) and formed on granitic glacial tills. The altitude of the sites was similar (300 – 400 m above sea level); mean annual temperatures on the sites average ~1°C, with approximately 570 mm of precipitation annually. Background rates of nitrogen (N) deposition are low (< 2 kg ha^{-1} yr^{-1}). The sites were characterised by the dominance of Scots Pine (*Pinus sylvestris* L.) and a feather moss ground layer (primarily *P. schreberi*) that increased in % cover with TSF. The feather moss layer is home to populations of Cyanobacteria (primarily *Nostoc* sp.) that play a key role in the boreal N cycle [11]. Dwarf shrub cover on the sites also increased with TSF. *Vaccinium myrtillus* L. and *V. vitis-idaea* L. were the dominant shrubs on young sites, but were replaced with ericaceous species (*Empetrum hermaphroditum* (Lange) Hagerup, *Calluna vulgaris* L.) as TSF increased.

Table 1

SAMPLING

Pleurozium schreberi shoots from five sites (abbreviated as RUS, LAD, GUO, TJA and REV: see Table 1) were analysed. We randomly selected shoots from six locations along an established

transect on each site (10-15 shoots/location); the shoots were consistently 5-7 cm in length. The shoots were placed in sterile plastic sample bags and stored at -20°C shortly after collection. The collected material comprised both green and brown sections of the moss shoot. The brown sections were attached to the green tips and were largely intact (i.e. they retained recognisable leaves).

EDAPHIC FACTORS AND FEATHER MOSS LAYER THICKNESS

In addition to sampling moss shoots, we also collected samples of the (mainly organic) material underlying the moss layer, in order to characterise edaphic conditions on each site. Samples were taken from twelve 1 m^2 plots, arranged in 100 – 300 m long transects. Five 2.5 cm diameter cores were collected a short distance (within ~1 m) from each plot. The moss/litter layer was removed from each core, which was then divided into a humus layer and mineral soil component (only the humus layer is considered in this study because we were most interested in conditions in the immediate proximity of the mosses). The five subsamples were then bulked to create a single sample for each plot, sealed in plastic bags and kept refrigerated (5°C) until they were analysed. Through-fall N (NH_4^+ - N and NO_3^- - N) was monitored over the period of a year (June 2012 – June 2013) using through-fall collectors as described in Rousk et al. [34].

Our analysis focused on edaphic factors that have been demonstrated to influence the structure of SMCs, on the assumption that similar factors would structure the microbial community of the moss layer. Particular emphasis was placed on N status, as boreal ecosystems are generally N-limited, and burning has been demonstrated to influence levels of soil N. Hence, we collected data on the following characteristics of the humus layer: total C & N, dissolved organic C (DOC), total dissolved N (TDN), extractable inorganic N (EIN), dissolved organic N (DON), through-fall N and the pH of the humus layer.

Total C and N were determined by dry combustion using a C elemental analyzer (Leco Corp, St Joseph, MI, USA), following drying of the humus samples at 60°C. In order to establish DOC and TDN, 5 g (dry weight equivalent, DW) of humus was placed in 25 ml of 0.05 M K_2SO_4, shaken for 30 min and centrifuged at 3000 g for 5 min. The K_2SO_4 extracts were 10-fold diluted with deionized water and analysed using a Shimadzu TCV-TNM1 analyser (Shimadzu Corp., Kyoto, Japan). Extractable

inorganic N (EIN) was also determined using a Shimadzu TCV-TNM1 analyser, after placing 5 g (DW) of humus in 25 ml 1M KCl, shaking the mixture for 30 min and centrifuging at 3000 g for 5 min. DON was calculated as TDN – EIN. Humus pH was established with an electronic pH meter after suspending 1 g (DW) of humus in 25 ml 0.01 M $CaCl_2$.

The thicknesses of a) the green and brown parts of the moss layer and b) the humus layer were recorded at each forest site in order to identify changes in the structure of the moss layer with TSF [49]. The brown moss tissue was distinguished from the humus layer according to structural integrity and degree of decomposition: if dead tissue was clearly attached to green tissue, it was classified as 'brown'. If the dead tissue was detached, obviously decomposed and/or fragmented, it was considered part of the humus layer. On each site, depth of moss (green and brown parts) and humus layer were recorded in twelve 1 m^2 plots along 100 – 300 m long transects.

MOLECULAR ANALYSIS

DNA was extracted and cleaned with a MoBio Powersoil kit (MoBio Laboratories Inc, Carlsbad, CA) in accordance with the manufacturer's instructions. Two whole shoots (comprising both green and brown sections) were placed in each reaction tube (30 reactions in total). Aliquots (15 µl) were taken from each DNA extract and pooled according to site i.e. five pooled samples were sequenced. The pooled samples were then analysed via tag-encoded FLX amplicon pyrosequencing, utilising a Roche 454 FLX instrument (454 Life Sciences, Branford, CT). The primer set 104F (5´-GGACGGGTGAGTAACACGTG-3´), 530R (5'-GTATTACCGCGGCTGCTG-3') was used for bacteria; the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used for fungi (i.e. the fungal dataset was primarily based on a ~700 bp region spanning the ITS1, 5.8S and ITS2 regions). The pyrosequencing was performed by Molecular Research LP (MR DNA, Shallowater, TX) based upon their published protocols.

The number of Cyanobacterial reads generated by the 104F-530R assay was found to be low. To check the analysis, a second pyrosequencing assay was performed using a different moss shoot sample and a primer set targeted at Cyanobacteria i.e. CYA359F (5'-GGGGAATYTTCCGCAATGGG-3') and 781R (5'-GGGGAATYTTCCGCAATGGG-3') [50]. To avoid flooding the sample with moss

chloroplasts, we immersed the moss shoots in 2 ml sterile water, agitated the sample, and performed the extraction on 750 µl of the supernatant. The PCR conditions were the same as those used for the first bacterial assay (i.e. utilising the primer set 104F-530R).

PROCESSING OF MOLECULAR DATA

Mothur 1.32.1 [51] was used to process raw sequence data generated by the amplicon pyrosequencing, following the pipelines described in Cutler et al. [24].

Bacterial 16S rRNA and fungal ITS gene flow files were trimmed and denoised with the mothur implementation of PyroNoise [52]. Bacterial sequences were aligned using the corresponding SILVA reference alignments [53] and only sequences spanning the targeted regions were kept. Data were denoised by clustering together sequences with 1 bp mismatch per 100 bp, and chimeras were removed using the mothur implementation of uchime [54]. Bacterial rRNA sequences were classified against the SILVA reference databases using the Wang method [55], with a cutoff value of 60% for taxonomic assignment. Bacterial sequences were also clustered into operational taxonomic units (OTUs) at the 97% similarity level, which corresponds approximately to the species level.

For fungal ITS sequences, following denoising, the ITS1 region was extracted using the ITS Extractor tool on the PlutoF Workbench [56,57] and sequences shorter than 100 bp were discarded. Chimeras were removed using the mothur implementations of uchime. Sequences were clustered into OTUs at a 93% similarity level, based on the average sequence divergence between named species in GenBank [58]. For OTU clustering, a distance matrix was constructed in mothur using pairwise distance values, with consecutive gaps treated as one and ignoring gaps at the end of pairs. Sequences were classified against the UNITE+INSDC fungal ITS database [59], modified as previously described [24], with a cutoff value of 50% for taxonomic assignment.

All sequence data were uploaded with MIMARKS-compliant metadata to the NCBI Sequence Read Archive under Bioproject number PRJNA287796.

STATISTICAL ANALYSIS

Microbial community structure was analysed using canonical correspondence analysis (CCA). Highly-correlated edaphic variables were manually removed to progressively simplify the model (refer to Online Resource 1 for further details). An ANOVA-like permutation test (using 100 permutations) was used to test the significance of the resulting CCA. Random sampling was used to standardise the number of reads in each sample, to ensure comparability. Individual-based rarefaction curves were produced to assess the impact of standardisation on the number of OTUs in each sample. As most environmental microbial communities are under-sampled, we also extrapolated the OTU data to estimate the 'true' underlying richness of the samples using the Chao 1 metric [60]. Shannon diversity was then calculated using the standardised datasets (including singletons). A Mantel test was used to assess the correlation between matrices of geographical distance (calculated from the coordinates of each site) and community dissimiliarity metrics (Bray-Curtis dissimilarity). The significance of the Mantel statistics was evaluated by randomly permuting the rows and columns of the first dissimilarity matrix (100 permutations). Regression analysis was used to test whether edaphic properties, through-fall N and moss layer thickness varied with TSF. We fitted regression models ranging from linear to a degree-3 polynomial to determine the simplest model sufficient to explain the relationship between each variable and TSF [61]. We performed ANOVA to compare the regressions, using an F-test [61]. A Bonferroni adjustment was performed on p-values with the significance level set at $p < 0.001$. All analyses were performed in R unless specified otherwise [62].

RESULTS

EDAPHIC FACTORS AND MOSS THICKNESS

The humus layer was acidic: pH ranged from 2.9 – 4.1, with the highest mean value (pH = 3.5) recorded for the GUO site (Table 2). The pH of the humus layer did not vary systematically with TSF. Accumulation of C and N (t ha^{-1} in the humus soil) were positively correlated with TSF but decreased on the oldest site (Fig. 1a; N not shown). The total C:N ratio of the humus layers stayed relatively constant at ~45, with the exception of the LAD site, where low total N resulted in a mean C:N ratio of 99.4±2.6. DOC and TDN were also found to be positively correlated (Kendall's rank correlation, tau = 0.72, $p < 0.001$). The highest values of DOC were recorded on sites of an intermediate age, resulting

in a humped relationship when plotted against TSF (Fig. 1b). EIN was positively correlated with TSF (Kendall's rank correlation, tau = 0.30, $p < 0.03$) but was also somewhat lower on the oldest site (Fig. 1c). Through-fall N did not vary systematically with time for the sites sampled in this study (Fig. 1d).

The length of the green portion of the moss layer stayed constant with TSF (mean length 2.4±0.1 cm) whereas the length of the brown tissue increased with TSF (Fig. 1e). The depth of the humus layer was also positively correlated with TSF, although it was somewhat shallower on the oldest site, REV (Fig. 1f).

Fig. 1

Table 2

MICROBIAL COMMUNITY STRUCTURE

A number of potential constraining variables were omitted from the analysis due to high levels of collinearity (Online Resource 1). The ordination of bacterial community constrained by TSF and pH (humus) was significant ($p = 0.025$) and explained about 62% of the variance in the bacterial community. The constraining variables were of similar overall significance (Fig. 2). The CCA biplot showed a clear separation of the youngest site, RUS, on an age gradient and the differentiation of the older sites along a pH gradient, with GUO, the site with the highest mean pH, as an outlier. When CCA was performed on the bacterial communities of the older sites (i.e. omitting RUS), with a) TSF and pH and b) pH only as constraining variables, the results were not significant. The Mantel correlation between matrices of geographical separation and community dissimilarity was not significant, either (Mantel correlation = 0.32, $p = 0.37$).

Fig. 2

For the fungal sites, the CCA also included TSF and pH as constraining variables (Fig. 3). The model was highly significant ($p < 0.01$) and explained about 64% of the variance in the data. Axis CCA1 was closely correlated with TSF; CCA2 was correlated with pH. As with the bacterial community, the CCA biplot showed a clear separation of the youngest site along CCA1 and the differentiation of the older

sites along a pH gradient. The CCA that excluded the RUS site was not significant with TSF and pH as constraining variables. However, CCA with only pH as a constraining variable was significant for the older sites ($p = 0.04$). In this analysis, pH explained 45% of the variance. There was no significant correlation between geographical separation and fungal community dissimilarity (Mantel correlation = 0.89, $p = 0.08$).

Fig. 3

BACTERIAL COMMUNITIES

Amplicon pyrosequencing generated 12336 bacterial reads, grouped into 895 OTUs. The number of reads per sample varied from 1215-4253 (samples were standardised to 1215 reads). Rarefaction analysis indicated that the sites had not been sampled to the point of saturation (Online Resource 2): observed richness in the standardised samples ranged from 48-61% of estimated community richness (Online Resource 3).

The bacterial communities on each site were highly uneven, with a few dominant taxa and many rare OTUs. Bacterial diversity was similar on each of the sites and did not appear to vary systematically with TSF (Table 3), nor was it obviously correlated with edaphic factors that are commonly thought to structure bacterial communities e.g. C:N and pH.

A total of nine bacterial phyla were identified, but only three of these were represented by significant numbers of reads: Proteobacteria (64% of all reads), Acidobacteria (29%) and Actinobacteria (5%). Most of the Proteobacterial reads were associated with the Gammaproteobacteria (Table 4). Prominent Gammaproteobacterial taxa included *Pseudomonas* sp. and *Serratia* sp. Sequences associated with the Betaproteobacteria were also abundant; most came from the genus *Burkholderia*. Most Acidobacterial sequences were *Edaphobacter* sp. Total numbers of Acidobacterial sequences appeared to decline with TSF (Fig. 4); Proteobacterial abundance exhibited the opposite pattern. The Actinobacterial reads were dominated by the family Actinomycetales.

The bacterial assay using the 104F-530R primer set revealed very few sequences that could be confidently associated with Cyanobacteria. The second assay utilising a targeted primer set identified more Cyanobacterial reads, but the level of taxonomic resolution was low. The sequences from the second assay were dominated by *Nostoc* sp. and mostly associated with the older sites (TJA in particular).

Table 3

Table 4

Fig. 4

FUNGAL COMMUNITIES

The analysis generated a total of 64647 fungal reads (10143 – 25854 per sample) before standardisation, encompassing 1162 OTUs. The number of reads was standardised to 10143 per sample. The 839 OTUs present in the standardized samples were more-or-less evenly distributed between the sites (Table 3). As with the bacterial assay, rarefaction analysis suggested that none of the communities had been sampled to saturation. There was also some crossing of the rarefaction curves, indicating that richness in the standardised samples might not reliably indicate underlying community diversity (Online Resource 2). Chao 1 estimates indicated that observed richness in the standardised samples represented 39-66% of extrapolated community richness (Online Resource 3). The ordering of the sites according to estimated richness differed from ranking based on the standardised samples. However, when the error of the estimates was taken into account, richness was similar on four of the five sites (estimated richness was markedly lower on the GUO site: Online Resource 3). Fungal diversity was generally higher than bacterial diversity.

Only three fungal phyla were abundant: the Ascomycota, Basidiomycota and Zygomycota (Table 5). Reads from the Ascomycota accounted for 62% of the total. The Basidiomycota accounted for 23% and Zygomycota 3.8%. Approximately 11% of reads could not be matched to databased sequences at a phylum level.

Table 5

Fungi from the class Leotiomycetes were by far the most common Ascomycetes: they were mostly from the family Helotiales (the genus *Crocicreas* was prominent). The class Sordariomycetes was also abundant: prominent members included *Hypocrea avellanea* and *Pestalotiopsis* sp. However, a substantial minority of reads (~19%) were unclassified below sub-phylum level.

Most of the Basidiomycetes were from the class Agaricomycetes, although few could be resolved to finer taxonomic levels. *Mycena* sp. (saprotrophs from the order Agricales) and *Clavulina* species (a putative mycorrhizal fungus from the order Cantherellales) were notably abundant on the youngest site (RUS). Reads from the class Tremellomycetes were also relatively abundant, notably *Cystofilobasidium* sp. and *Cryptococcus* sp. The class Microbotryomycetes was mainly represented by *Rhodotorula* sp. (pigmented yeasts).

Where the level of taxonomic resolution was suitably high, taxa were tentatively assigned to ecological groups, based on published literature (ultimately, about a third of fungal sequences were resolved to at least genus level). A variety of different lifestyles were represented, including saprotrophs, endophytes/plant parasites and mycorrhizal fungi. Saprotrophic fungi, notably *Mortierella* sp., *Crocicreas* sp., *Aureobasidium* sp. and a variety of yeasts (*Cystofilobasidium* sp., *Cryptococcus* sp., *Rhodoturula* sp.) were dominant on sites of all ages. The saprotrophic fungi encompassed a range of 'early' and 'late' varieties, as classified by previous studies of fungal succession. For example, Ascomycetes from the Dothideomycetes (e.g. *Aureobasidium* and *Cladosporium* spp.) are often considered early/primary colonists; *Mortierella* is considered a late/secondary variety [31,63,64]. Reads associated with mycorrhizal fungi were relatively uncommon throughout and appeared to decline with time since burning (Table 6). All the putative mycorrhizal taxa identified were ectomycorrhizae.

Table 6

DISCUSSION

The analysis of the sequencing data indicated temporal changes in both the bacterial and fungal communities of the bryosphere. Variations in community structure could be largely explained with just two constraining variables, TSF and pH of the humus layer. However, the significance of TSF was largely driven by the youngest site, RUS, which was an outlier in terms of microbial community composition. Shifts in community structure were accompanied by changes in life history strategy: copiotrophic bacteria became dominant as TSF increased, and mycorrhizal fungi declined in relative abundance.

EDAPHIC FACTORS AND MOSS THICKNESS

Overall, C and N appeared to accumulate in the moss layer over time. This trend is consistent with well-established models of ecological succession [65]. The LAD site was located in a small forest stand and a more pronounced edge effect (e.g. more frequent desiccation) may have been less favourable for N fixation, resulting in a higher C:N ratio. Most models of forest succession predict decreased soil pH over time as organic acids accumulate [44]. This was not observed in the moss layer, possibly because organic acids were leached from the base of the moss layer into the mineral soil (an increase in mineral soil pH with TSF was recorded (results not shown)). The moss layer data were also predictable: the length of the photosynthesising portion of the shoot stayed constant whilst more recalcitrant C accumulated in brown tissue and the underlying humus layer.

MICROBIAL COMMUNITY STRUCTURE

The ordinations indicated that TSF was an important factor in explaining microbial community structure. Strictly speaking, time is not an environmental factor; rather, it integrates an array of changes, both biotic and abiotic, that occur over time. In this case, TSF was closely related to several edaphic factors (notably total C, total N and EIN) and the physical attributes of the bryosphere (moss layer thickness and moss litter accumulation). Progressive changes in these factors are likely to have impacted on microbial community structure. However, the chronosequence had few young sites, and this made it difficult to infer microbial community dynamics in the earliest stages of succession. A future study might usefully focus on the 121-year period separating the two youngest sites, as it is

likely that many changes occurred early in the recovery of the ecosystem. One of the most striking features of the CCA plots was the isolation of the youngest site, RUS (14 years since burning) from the older sites (TSF > 100 years). The distinct community on this site may well have been due the relatively early successional stage (i.e. differences in canopy structure, light availability, moisture regime, etc.) This outlier increased the significance of TSF: it is likely that the importance of this factor declines for mid- and late successional sites, as evidenced by the CCA performed on only the older sites.

Previous studies have reported that pH plays a key role in structuring SMCs [27-29], including those in boreal locations [13,17,20,21]. In the present study, CCA indicated that pH was important to the microbes of the bryosphere, too, particularly those on the older sites. In fact, pH was nearly as important as TSF in terms of accounting for community structure, reinforcing the link between the attributes of the bryosphere and edaphic properties. As with TSF, it is likely that pH is a proxy for other biochemical properties (e.g. the availability of mineral nutrients or cytotoxic Al^{3+}), rather than a proximate cause of community variation [20]. After rapid changes in the early stages of secondary succession, factors such as slight variations in pH become more significant than TSF in determining microbial community structure (particularly fungi).

BACTERIAL COMMUNITIES

The most common bacterial taxa occurred on all of the sites: community turnover was relatively low. However, the bacterial ordinations suggest that the youngest site (RUS) was distinct from the older sites in terms of community composition. It is possible that bacterial communities either resist fire-induced changes or assemble and equilibrate relatively quickly (years-decades) after burning. Xiang et al. [17] observed that soil dwelling bacteria in a boreal soil were relatively insensitive to fire disturbance, and recovered to pre-fire levels within 11 years of burning. Bergner et al. [66] also commented on the robustness of soil bacteria in the face of fire disturbance.

At a phylum level, the bacterial communities of the bryosphere were broadly similar to those described for boreal soils. A number of studies have reported the dominance of Proteobacteria in boreal soils along with high relative abundances of Acidobacteria and Actinobacteria [21,67,68]. Smith et al. [13]

reported that Betaproteobacteria were highly characteristic in boreal soils one year after fire; Alpha- and Gammaproteobacteria were associated with unburnt treatments. Similarly, Xiang et al. [17] noted an increase in the relative abundance of Betaproteobacteria after burning and a concomitant decrease in Alphaproteobacteria. In our study, Gammaproteobacteria certainly appeared to be associated with sites that had recovered from burning (high numbers of reads on mid- to late-successional sites). However, Betaproteobacterial reads increased with TSF, whilst Alphaproteobacterial reads decreased. The timescales involved in our study were much longer than those in published accounts and transient changes in Alpha-/Betaproteobacterial communities that occurred immediately after burning would have been missed. A sparsity of reads, coupled with poor taxonomic resolution, meant it was not possible to comment on changes in Cyanobacterial community.

An absence of plant roots (and the exudates that can support dense concentrations of bacteria), periods of desiccation and exposure to UV radiation might all be expected to imposed stringent environmental filters on bacterial colonisation in the moss layer, with the net effect of suppressing diversity. However, Shannon diversity metrics for the bacterial communities (3.6 – 4.6) fell within the range previously reported for boreal soil communities. Dimitriu and Grayston [20], for example, recorded bacterial Shannon diversity figures around 3.5 (range 2.9 – 5.8) in boreal Canada; Neufeld and Mohn [68] observed values ~5. Bacterial diversity was probably enhanced by the synchronous sampling of two distinct niches (living and senescent tissue). Rarefaction analysis indicated that the communities had not been sampled to saturation, i.e. bacterial *richness* was underestimated. However, the Chao 1 estimates suggested that richness was consistently underestimated in the standardised samples. Overall, there was no overriding pattern in diversity with TSF.

Changes in the relative abundance of the main bacterial phyla with TSF were suggestive of systematic shifts in life history strategy. Proteobacterial reads generally increased with TSF; Acidobacterial sequences exhibited a corresponding decrease (Fig. 4). Proteobacteria often adopt copiotrophic strategies where resources are plentiful [69]. In contrast, studies suggest that Acidobacteria are capable of metabolising a range of substrates, including moderately recalcitrant to recalcitrant compounds such as hemicellulose, cellulose, and chitin [70]. As such, they are often associated with oligotrophic conditions and should be able to survive in habitats unfavourable to copiotrophs [30]. A

similar trend has also been observed in bryophytic soil crusts where high C availability was related to a higher abundance of Bacteroidetes compared to Acidobacteria [4]. Therefore, an increase in Proteobacterial abundance, coupled with a decline in reads from Acidobacteria, may indicate ameliorating growth conditions for bacteria with time and an overall shift from K- to r-strategies as plant succession progresses and carbon and nitrogen accumulate in the system [71]. This was at odds with our original expectation of a shift from r- to K-selected bacterial life history strategies.

FUNGAL COMMUNITIES

As with the bacteria, the ordinations suggested that the youngest site (RUS) was qualitatively different from the older sites in terms of its fungal community. A number of authors have noted the sensitivity of fungal communities to burning [13,16,66] and forestry practices [72]. However, even though fungi are sensitive to disturbance, and slower than bacteria to recover, fungal communities are likely to return to a pre-fire state within a few decades of disturbance. LeDuc et al. [18], for example, observed few changes in ectomycorrhizal communities on sites >25 years old in a fire chronosequence. Similarly, Treseder et al. [73] found that ECM in the Alaskan boreal forest returned to pre-fire levels within 15 years and Holden et al. specified a fungal recovery period of at least 24 years [16]. Davey et al. [72] reported the recovery of fungal communities associated with boreal feather mosses within 30 years of clear-cutting.

Based on our findings and other published reports, it is likely that the re-establishment of fungal communities in the moss layer, either from spores or hyphae surviving in the soil, is rapid in successional terms [years or decades: see 15]: most of the major structural changes on our sites occurred early on. The recovery of the fungal community may have been related to the recovery of the moss layer [which takes 20-30 years: 46] and/or the re-establishment of understorey herbs and shrubs [19,25,74]. If burning of the forest becomes more frequent, i.e. if the return period of fires becomes less than the recovery period of the fungal community, fungi might be suppressed and the microbial community could be kept in a perpetually early stage of succession. Restraint on the development of fungal taxa could have implications for the cycling of recalcitrant C in the moss layer.

Ascomycetes are often the dominant fungi in soil habitats. The ratio of Ascomycetes to Basidiomycetes in the moss layer was similar to the figures reported for soil fungi. Hartmann et al. [67], for example, reported an Ascomycete:Basidiomycete ratio of 2:1 in the soil of a coniferous forest in British Columbia. Davey et al. [25,75] recorded ratios of 1.5 – 2:1 in studies of boreal mosses. Many of the fungal taxa observed were familiar from soil habitats elsewhere. Davey et al. [75], for example, recorded the dominance of Basidiomycetes from the order Agaricales in a survey of boreal moss microbes. *Mortierella*, a genus that is reported in many studies of forest soil microfungi [e.g. 19,23,76], was also common on the study sites, along with a number of hyphomycetes and yeast-like fungi previously reported from forest litter e.g. *Cystofilobasidium*, *Cryptococcus* and *Rhodotorula* spp. [77]. Other fungi commonly recorded in comparable boreal habitats (notably *Penicillium*, *Trichoderma* and *Umbelopsis* spp.) were much less abundant. Furthermore, taxa more usually associated with the phyllosphere were common on some sites, e.g. *Pestalotiopsis* spp. (usually found on pine cones and needles) and *Cercospora* spp. (plant pathogens that cause leaf spots). Other researchers have reported the role that the bryosphere plays in the interception of litter from vascular plants in the forest canopy and understory [78]. The bryosphere can therefore be characterised as an interface between aboveground and litter/soil habitats, with characteristics of both. Such a variety of ecological niches may well promote fungal diversity in this habitat.

The youngest site (RUS) was characterised by the abundance of two saprotrophic taxa, *Mycena* sp. and *Crocicreas* sp., which were rare on older sites. Reads associated with *Mortierella* sp. and *Trichoderma* sp., were rare on the RUS site and most abundant on the two oldest sites. These taxa are often considered ‘late’ fungal colonists i.e. they are usually associated with well-decomposed organic matter. An increase in the relative abundance of ‘late’ fungal colonists on older sites might reflect a higher proportion of senescent material in the moss layer. Similarly, the rarity of hyphomycetes on RUS might also be indicative of systematic difference in litter quality between the youngest site and locations > 100 yrs old.

The predominance of saprotrophic fungal taxa was consistent with previous studies conducted in boreal forests. The composition of the mycorrhizal community was also as expected: trees and shrubs in boreal forests often exhibit an obligate need for ericoid and ectomycorrhizal fungi [79]. Some

mycorrhizal fungi can penetrate the moss layer from below in boreal forests [75] and appeared to have done so in this study, although it was not possible to distinguish between active and inactive cells. However, ECM fungi were greatly outnumbered by saprotrophs and non-mycorrhizal root endophytes and there wasn't any evidence for a shift from saprophytic to mycorrhizal fungi consistent with our expectations. Indeed, the opposite pattern was observed: the number of sequences from putative mycorrhizal taxa was highest on the youngest site and declined thereafter. This might not be representative of the boreal mycorrhizal community generally. For example, it might result from a decline in MR species that forage in the moss layer, or a general decrease in the exploitation of moss as a substrate. However, it could be associated with important changes in the bryosphere associated with succession.

The decline in the proportion of reads associated with mycorrhizae could represent either an absolute decline (MR biomass decreases) or relative decline (MR biomass stays the same, but other fungi proliferate) in mycorrhizal abundance in the bryosphere. An absolute decline could be driven by changes in nutrient status or biotic factors. Nitrogen availability has been implicated in changes in mycorrhizal communities: for example LeDuc et al. [18] found that changes in mycorrhizal community structure related to the availability of DON and free amino-acid N in soil. Högberg et al. [80] hypothesised that ECM might decline in abundance as N concentrations increase and plants allocate less C belowground. Usually, N increases during succession as organic matter accumulates in the system [65]. Clear temporal trends in N availability were not generally apparent in our study, but the RUS site, which had the highest number of reads associated with putative mycorrhizal fungi, was characterised by the lowest figures for TDN and DON (possibly due to the thinness of the humus layer on this site). It is possible that mycorrhizal mycelia diminished as N accumulated during succession.

Changes in biotic factors may also have impacted on the mycorrhizal community. For example, Davey et al. [72] reported that stressed feather mosses in young forest stands (<5 years since clear-cutting) were readily colonised by mycorrhizal fungi. The abundance of these fungi would be expected to decline as growth conditions for the feather mosses ameliorated over time. Compositional changes in the bacterial community might also drive a decline in mycorrhizal biomass. The abundance of Proteobacteria within the genera *Burkholderia* and *Serratia* increases with TSF and these bacteria

have been demonstrated to restrict the growth of plant pathogens (notably fungi) on some occasions [81-84]. It is possible that these bacteria are antagonistic towards fungi in the boreal moss layer and suppressed colonisation by mycorrhizae [85,86], although the presence of bacteria such as *Burkholderia* may be positive in certain circumstances [e.g. when they act as 'helper bacteria': 87].

As with the bacteria, fungal richness in the standardised samples was underestimated. There was some variation in the shape of the rarefaction curves; however, the Chao 1 estimates indicated that fungal richness was broadly similar on four of the five sites and not clearly related to TSF. The Shannon diversity figures told a similar story. The low estimated richness of the GUO site was probably connected with an extremely uneven distribution of reads between OTUs (Table 3).

Unusually, fungal diversity was comparable to, or higher than, bacterial diversity (although still within the range observed by Holden et al. [16] across a 100-year fire chronosequence in the Alaskan boreal forest). It should be noted that the number of reads used to calculate fungal diversity was much higher than the equivalent figure for bacteria (10143 vs 1215, respectively). Nevertheless, the factors that mitigate against bacterial growth e.g. desiccation, low pH, exposure to UV radiation and recalcitrant C compounds, are less limiting for fungi. A variety of substrates (from both the moss layer and vascular plant layers) may promote fungal diversity. It is also known that fungal communities vary with depth within the moss layer [64], with different communities in green and brown tissue. Along-stem variation in substrate quality might increase fungal diversity, while bacterial diversity decreases with depth along the moss stem (Arróniz-Crespo et al. unpublished results).

Spatial segregation might also explain the seemingly contradictory development of the bacterial and fungal communities over time. Changes in the bacterial community suggest an amelioration of growth conditions, but this occurs alongside increasing fungal dominance. This pattern is explicable if the bacteria responded to changes in green tissue (which remained at a constant length), but the fungal community was primarily influenced by changes towards the base of the moss layer. An analysis that looked at along-stem variation in bacterial and fungal communities with TSF could resolve this issue.

CONCLUSIONS

Both the bacterial and fungal communities of the bryosphere were similar to those reported in studies of boreal SMCs. Taxonomic diversity was also comparable, even though the bryosphere is a potentially stressful habitat for microbes. Overall, the bryosphere appeared to be an interface between aboveground and belowground habitats and this configuration might promote fungal diversity. Our study provided evidence for post-fire changes in bacterial and fungal communities in the bryosphere. The youngest site (RUS, 14 years since burning) was qualitatively different from the older sites in terms of microbial community composition. Presumably, most of the major changes in community structure occurred during the post-fire recovery of the moss layer (20-30 years). Thereafter, the communities appeared to differentiate along a narrow pH gradient. In terms of community composition, the bacterial community shifted from oligotrophic to copiotrophic life history strategies, suggesting an amelioration in growth conditions. Fungal communities were dominated by saprotrophs, but there was an apparent decline in MR fungi with TSF, possibly related to changing N status. Evidence for the decadal-scale legacy of burning has implications for the ecology of boreal forests if, as predicted, the return period of wildfires decreases over the next century. Most notably, reduced fire return periods could keep the microbial communities of the bryosphere (particularly fungi) at an early successional stage, with a knock-on effect on the cycling of recalcitrant C.

LITERATURE CITED

1. Bragina A, Berg C, Mueller H, Moser D, Berg G (2013) Insights into functional bacterial diversity and its effects on Alpine bog ecosystem functioning. Scientific Reports 3. doi:10.1038/srep01955

2. Bragina A, Oberauner-Wappis L, Zachow C, Halwachs B, Thallinger GG, Mueller H, Berg G (2014) The *Sphagnum* microbiome supports bog ecosystem functioning under extreme conditions. Mol Ecol 23:4498-4510. doi:10.1111/mec.12885

3. Jassey VEJ, Chiapusio G, Binet P, Buttler A, Laggoun-Defarge F, Delarue F, Bernard N, Mitchell EAD, Toussaint M-L, Francez A-J, Gilbert D (2013) Above- and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions. Global Change Biol 19:811-823. doi:10.1111/gcb.12075

4. Moquin SA, Garcia JR, Brantley SL, Takacs-Vesbach CD, Shepherd UL (2012) Bacterial diversity of bryophyte-dominant biological soil crusts and associated mites. J Arid Environ 87:110-117. doi:10.1016/j.jaridenv.2012.05.004

5. Lindo Z, Gonzalez A (2010) The bryosphere: an integral and influential component of the Earth's biosphere. Ecosystems 13:612-627. doi:10.1007/s10021-010-9336-3

6. Turetsky MR, Bond-Lamberty B, Euskirchen E, Talbot J, Frolking S, McGuire AD, Tuittila ES (2012) The resilience and functional role of moss in boreal and arctic ecosystems. New Phytol 196:49-67. doi:10.1111/j.1469-8137.2012.04254.x

7. IPCC (2013) Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

8. Turetsky MR (2003) The role of bryophytes in carbon and nitrogen cycling. Bryologist 106:395-409. doi:10.1639/05

9. Vogel JG, Gower ST (1998) Carbon and nitrogen dynamics of boreal jack pine stands with and without a green alder understory. Ecosystems 1:386-400. doi:10.1007/s100219900032

10. Bond-Lamberty B, Gower ST (2007) Estimation of stand-level leaf area for boreal bryophytes. Oecologia 151:584-592. doi:10.1007/s00442-006-0619-5

11. DeLuca TH, Zackrisson O, Nilsson M-C, Sellstedt A (2002) Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419:917-920

12. Nasholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914-916. doi:10.1038/31921

13. Smith NR, Kishchuk BE, Mohn WW (2008) Effects of wildfire and harvest disturbances on forest soil bacterial communities. Appl Environ Microbiol 74:216-224. doi:10.1128/aem.01355-07

14. DeLuca TH, Sala A (2006) Frequent fire alters nitrogen transformations in ponderosa pine stands of the inland northwest. Ecology 87:2511-2522. doi:10.1890/0012-9658(2006)87[2511:ffanti]2.0.co;2

15. Kim Y-H, Kim IS, Moon EY, Park JS, Kim S-J, Lim J-H, Park BT, Lee EJ (2011) High Abundance and Role of Antifungal Bacteria in Compost-Treated Soils in a Wildfire Area. Microbial Ecology 62:725-737. doi:10.1007/s00248-011-9839-2

16. Holden SR, Gutierrez A, Treseder KK (2013) Changes in soil fungal communities, extracellular enzyme activities, and litter decomposition across a fire chronosequence in Alaskan boreal forests. Ecosystems 16:34-46. doi:10.1007/s10021-012-9594-3

17. Xiang X, Shi Y, Yang J, Kong J, Lin X, Zhang H, Zeng J, Chu H (2014) Rapid recovery of soil bacterial communities after wildfire in a Chinese boreal forest. Scientific Reports 4. doi:10.1038/srep03829

18. LeDuc SD, Lilleskov EA, Horton TR, Rothstein DE (2013) Ectomycorrhizal fungal succession coincides with shifts in organic nitrogen availability and canopy closure in post-wildfire jack pine forests. Oecologia 172:257-269. doi:10.1007/s00442-012-2471-0

19. De Bellis T, Kernaghan G, Widden P (2007) Plant community influences on soil microfungal assemblages in boreal mixed-wood forests. Mycologia 99:356-367. doi:10.3852/mycologia.99.3.356

20. Dimitriu PA, Grayston SJ (2010) Relationship between soil properties and patterns of bacterial beta-diversity across reclaimed and natural boreal forest soils. Microb Ecol 59:563-573. doi:10.1007/s00248-009-9590-0

21. Sun H, Terhonen E, Koskinen K, Paulin L, Kasanen R, Asiegbu FO (2014) Bacterial diversity and community structure along different peat soils in boreal forest. Applied Soil Ecology 74:37-45. doi:10.1016/j.apsoil.2013.09.010

22. Kernaghan G, Patriquin G (2011) Host Associations Between Fungal Root Endophytes and Boreal Trees. Microbial Ecology 62:460-473. doi:10.1007/s00248-011-9851-6

23. Summerbell RC (2005) Root endophyte and mycorrhizosphere fungi of black spruce, *Picea mariana*, in a boreal forest habitat: influence of site factors on fungal distributions. Studies in Mycology 53:121-145

24. Cutler NA, Chaput DL, van der Gast CJ (2014) Long-term changes in soil microbial communities during primary succession. Soil Biology and Biochemistry 69:359-370

25. Davey ML, Heegaard E, Halvorsen R, Ohlson M, Kauserud H (2012) Seasonal trends in the biomass and structure of bryophyte-associated fungal communities explored by 454 pyrosequencing. New Phytol 195:844-856. doi:10.1111/j.1469-8137.2012.04215.x

26. Osono T, Ueno T, Uchida M, Kanda H (2012) Abundance and diversity of fungi in relation to chemical changes in arctic moss profiles. Polar Science 6:121-131. doi:10.1016/j.polar.2011.12.001

27. Griffiths RI, Thomson BC, James P, Bell T, Bailey M, Whiteley AS (2011) The bacterial biogeography of British soils. Environ Microbiol 13:1642-1654. doi:10.1111/j.1462-2920.2011.02480.x

28. Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75:5111-5120

29. Tedersoo L, Bahram M, Polme S, Koljalg U, Yorou NS, Wijesundera R, Villarreal Ruiz L, Vasco-Palacios AM, Pham Quang T, Suija A, Smith ME, Sharp C, Saluveer E, Saitta A, Rosas M, Riit T, Ratkowsky D, Pritsch K, Poldmaa K, Piepenbring M, Phosri C, Peterson M, Parts K, Paertel K, Otsing E, Nouhra E, Njouonkou AL, Nilsson RH, Morgado LN, Mayor J, May TW, Majuakim L, Lodge DJ, Lee SS, Larsson K-H, Kohout P, Hosaka K, Hiiesalu I, Henkel TW, Harend H, Guo L-d, Greslebin A, Grelet G, Geml J, Gates G, Dunstan W, Dunk C, Drenkhan R, Dearnaley J, De Kesel A, Tan D, Chen X, Buegger F, Brearley FQ, Bonito G, Anslan S, Abell S, Abarenkov K (2014) Global diversity and geography of soil fungi. Science 346. doi:10.1126/science.1256688

30. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354-1364. doi:10.1890/05-1839

31. Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Hogberg P, Stenlid J, Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. New Phytologist 173:611-620. doi:10.1111/j.1469-8137.2006.01936.x

32. Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629-1633. doi:10.1126/science.1094875

33. Cutler N (2011) Nutrient limitation during long-term ecosystem development inferred from a mat-forming moss. The Bryologist 114:204-214

34. Rousk K, Rousk J, Jones DL, Zackrisson O, DeLuca TH (2013) Feather moss nitrogen acquisition across natural fertility gradients in boreal forests. Soil Biology & Biochemistry 61:86-95. doi:10.1016/j.soilbio.2013.02.011

35. DeLuca TH, Zackrisson O, Gentili F, Sellstedt A, Nilsson M-C (2007) Ecosystem controls on nitrogen fixation in boreal feather moss communities. Oecologia 152:121-130. doi:10.1007/s00442-006-0626-6

36. Jumpponen A (2003) Soil fungal community assembly in a primary successional glacier forefront ecosystem as inferred from rDNA sequence analyses. New Phytol 158:569-578. doi:10.1046/j.1469-8137.2003.00767.x

37. Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. J Ecol 98:725-736

38. Peltzer DA, Wardle DA, Allison VJ, Baisden WT, Bardgett RD, Chadwick OA, Condron LM, Parfitt RL, Porder S, Richardson SJ, Turner BL, Vitousek PM, Walker J, Walker LR (2010) Understanding ecosystem retrogression. Ecol Monogr 80:509-529. doi:10.1890/09-1552.1

39. Vitousek P, Asner GP, Chadwick OA, Hotchkiss S (2009) Landscape-level variation in forest structure and biogeochemistry across a substrate age gradient in Hawaii. Ecology 90:3074-3086. doi:10.1890/08-0813.1

40. Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305:509-513. doi:10.1126/science.1098778

41. Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK (2007) Microbial community succession in an unvegetated, recently deglaciated soil. Microb Ecol 53:110-122

42. Schütte UME, Abdo Z, Bent SJ, Williams CJ, Schneider GM, Solheim B, Forney LJ (2009) Bacterial succession in a glacier foreland of the High Arctic. ISME J 3:1258-1268

43. Sigler WV, Crivii S, Zeyer J (2002) Bacterial succession in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb Ecol 44:306-316

44. Matthews JA (1992) The ecology of recently-deglaciated terrain: a geoecological approach to glacier forelands and primary succession. Cambridge University Press, Cambridge

45. DeLuca TH, Nilsson MC, Zackrisson O (2002) Nitrogen mineralization and phenol accumulation along a fire chronosequence in northern Sweden. Oecologia 133:206-214. doi:10.1007/s00442-002-1025-2

46. Zackrisson O, DeLuca TH, Nilsson MC, Sellstedt A, Berglund LM (2004) Nitrogen fixation increases with successional age in boreal forests. Ecology 85:3327-3334. doi:10.1890/04-0461

47. Zackrisson O, Nilsson MC, Wardle DA (1996) Key ecological function of charcoal from wildfire in the Boreal forest. Oikos 77:10-19. doi:10.2307/3545580

48. Niklasson M, Granstrom A (2000) Numbers and sizes of fires: Long-term spatially explicit fire history in a Swedish boreal landscape. Ecology 81:1484-1499

49. Lagerstrom A, Nilsson MC, Zackrisson O, Wardle DA (2007) Ecosystem input of nitrogen through biological fixation in feather mosses during ecosystem retrogression. Funct Ecol 21:1027-1033. doi:10.1111/j.1365-2435.2007.01331.x

50. Nubel U, GarciaPichel F, Muyzer G (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl Environ Microbiol 63:3327-3332

51. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber

CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology 75:7537-7541. doi:10.1128/aem.01541-09

52. Quince C, Lanzen A, Curtis TP, Davenport RJ, Hall N, Head IM, Read LF, Sloan WT (2009) Accurate determination of microbial diversity from 454 pyrosequencing data. Nature Methods 6:639-U627. doi:10.1038/nmeth.1361

53. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Gloeckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41:D590-D596. doi:10.1093/nar/gks1219

54. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200. doi:10.1093/bioinformatics/btr381

55. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261-5267. doi:10.1128/aem.00062-07

56. Abarenkov K, Tedersoo L, Nilsson RH, Vellak K, Saar I, Veldre V, Parmasto E, Prous M, Aan A, Ots M, Kurina O, Ostonen I, Jogeva J, Halapuu S, Poldmaa K, Toots M, Truu J, Larsson K-H, Koljalg U (2010) PlutoF-a web based workbench for ecological and taxonomic research, with an online implementation for fungal ITS sequences. Evolutionary Bioinformatics 6:189-196. doi:10.4137/ebo.s6271

57. Nilsson RH, Veldre V, Hartmann M, Unterseher M, Amend A, Bergsten J, Kristiansson E, Ryberg M, Jumpponen A, Abarenkov K (2010) An open source software package for automated extraction of ITS1 and ITS2 from fungal ITS sequences for use in high-throughput community assays and molecular ecology. Fungal Ecology 3:284-287. doi:10.1016/j.funeco.2010.05.002

58. Hibbett DS, Ohman A, Glotzer D, Nuhn M, Kirk P, Nilsson RH (2011) Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences. Fungal Biology Reviews 25:38-47. doi:10.1016/j.fbr.2011.01.001

59. Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vralstad T, Liimatainen K, Peintner U, Koljalg U (2010) The UNITE database for molecular identification of fungi - recent updates and future perspectives. New Phytologist 186:281-285. doi:10.1111/j.1469-8137.2009.03160.x

60. Colwell RK, Coddington JA (1994) Estimating terrestrial biodiversity through extrapolation. Philos Trans R Soc Lond, Ser B: Biol Sci 345:101-118. doi:10.1098/rstb.1994.0091

61. James G, Witten D, Hastie T, Tibshirani R (2013) An introduction to statistical learning. Springer, New York

62. R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

63. Kasai K, Morinaga T, Horikoshi T (1995) Fungal succession in the early decomposition process of pine cones on the floor of *Pinus densiflora* forests. Mycoscience 36:325-334. doi:10.1007/bf02268608

64. Osono T, Trofymow JA (2012) Microfungal diversity associated with *Kindbergia oregana* in successional forests of British Columbia. Ecological Research 27:35-41. doi:10.1007/s11284-011-0866-8

65. Walker LR, del Moral R (2003) Primary succession and ecosystem rehabilitation. Cambridge University Press, Cambridge

66. Bergner B, Johnstone J, Treseder KK (2004) Experimental warming and burn severity alter soil CO2 flux and soil functional groups in a recently burned boreal forest. Global Change Biol 10:1996-2004. doi:10.1111/j.1365-2486.2004.00868.x

67. Hartmann M, Howes CG, van Insberghe D, Yu H, Bachar D, Christen R, Nilsson RH, Hallam SJ, Mohn WW (2012) Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests. ISME J 6:2199-2218. doi:10.1038/ismej.2012.84

68. Neufeld JD, Mohn WW (2005) Unexpectedly high bacterial diversity in arctic tundra relative to boreal forest soils, revealed by serial analysis of ribosomal sequence tags. Appl Environ Microbiol 71:5710-5718. doi:10.1128/aem.71.10.5710-5718.2005

69. Kirchman DL (2012) Processes in Microbial Ecology. OUP, Oxford

70. Jones RT, Robeson MS, Lauber CL, Hamady M, Knight R, Fierer N (2009) A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. ISME J 3:442-453. doi:10.1038/ismej.2008.127

71. Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environmental Microbiology 12:1842-1854. doi:10.1111/j.1462-2920.2010.02189.x

72. Davey ML, Kauserud H, Ohlson M (2014) Forestry impacts on the hidden fungal biodiversity associated with bryophytes. FEMS Microbiol Ecol 90:313-325. doi:10.1111/1574-6941.12386

73. Treseder KK, Mack MC, Cross A (2004) Relationships among fires, fungi, and soil dynamics in Alaskan Boreal Forests. Ecol Appl 14:1826-1838. doi:10.1890/03-5133

74. Davey ML, Tsuneda A, Currah RS (2010) Saprobic and parasitic interactions of *Coniochaeta velutina* with mosses. Botany-Botanique 88:258-265. doi:10.1139/b10-004

75. Davey ML, Heegaard E, Halvorsen R, Kauserud H, Ohlson M (2013) Amplicon-pyrosequencing-based detection of compositional shifts in bryophyte-associated fungal communities along an elevation gradient. Molecular Ecology 22:368-383. doi:10.1111/mec.12122

76. Singh P (1976) Some fungi in forest soils of Newfoundland. Mycologia 68:881-890. doi:10.2307/3758804

77. Slavikova E, Vadkertiova R (2000) The occurrence of yeasts in the forest soils. Journal of Basic Microbiology 40:207-212. doi:10.1002/1521-4028(200007)40:3<207::aid-jobm207>3.3.co;2-8

78. Jackson BG, Nilsson M-C, Wardle DA (2013) The effects of the moss layer on the decomposition of intercepted vascular plant litter across a post-fire boreal forest chronosequence. Plant Soil 367:199-214. doi:10.1007/s11104-012-1549-0

79. Bardgett RD, Wardle DA (2010) Aboveground-Belowground Linkages. Oxford University Press, Oxford

80. Hogberg MN, Hogberg P, Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia 150:590-601. doi:10.1007/s00442-006-0562-5

81. Dumas MT (1992) INHIBITION OF ARMILLARIA BY BACTERIA ISOLATED FROM SOILS OF THE BOREAL MIXEDWOOD FOREST OF ONTARIO. European Journal of Forest Pathology 22:11-18

82. Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097-1100. doi:10.1126/science.1203980

83. Opelt K, Berg G (2004) Diversity and antagonistic potential of bacteria associated with bryophytes from nutrient-poor habitats of the Baltic Sea coast. Applied and Environmental Microbiology 70:6569-6579. doi:10.1128/aem.70.11.65-69.2004

84. Opelt K, Chobot V, Hadacek F, Schoenmann S, Eberl L, Berg G (2007) Investigations of the structure and function of bacterial communities associated with Sphagnum mosses. Environmental Microbiology 9:2795-2809. doi:10.1111/j.1462-2920.2007.01391.x

85. Bending GD, Poole EJ, Whipps JM, Read DJ (2002) Characterisation of bacteria from Pinus sylvestris-Suillus luteus mycorrhizas and their effects on root-fungus interactions and plant growth. FEMS Microbiology Ecology 39:219-227. doi:Pii s0168-6496(01)00215-x 10.1111/j.1574-6941.2002.tb00924.x

86. Shcherbakov AV, Bragina AV, Kuzmina EY, Berg C, Muntyan AN, Makarova NM, Malfanova NV, Cardinale M, Berg G, Chebotar VK, Tikhonovich IA (2013) Endophytic

bacteria of Sphagnum mosses as promising objects of agricultural microbiology. Microbiology 82:306-315. doi:10.1134/s0026261713030107

87. Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22-36. doi:10.1111/j.1469-8137.2007.02191.x

TABLES

Table 1: Summary information for sampling locations.

Site name	Site code	Location	Time since fire (yrs)
Rusklidtjärns	RUS	64° 47' 49" N 18° 41' 46" E	14
Laddock	LAD	65° 56' 43" N 18° 22' 37" E	135
Guorbäive	GUO	65° 48' 57" N 19° 02' 54" E	182
Tjadness	TJA	65° 47' 28" N 18° 43' 52" E	255
Reivo	REV	65° 46' 28" N 19° 06' 19" E	366

Table 2: Edaphic factors for the five boreal forest sites. The analysis was performed on the humus layer. The figures presented for mean pH are derived arithmetically, but the values are almost identical to the geometric means. Values represent means ± SE (n = 12).

Site	Total N (t ha^{-1})	TDN (mg kg^{-1})	C:N	pH
RUS	0.3 ± 0.03	39.4 ± 7.6	44.6 ± 1.6	3.30 ± 0.04
LAD	0.1 ± 0.01	120.7 ± 15.9	99.4 ± 2.6	3.06 ± 0.02
GUO	0.4 ± 0.01	NA	45.1 ± 1.4	3.48 ± 0.18
TJA	0.5 ± 0.01	95.4 ± 1.9	43.5 ± 0.3	2.98 ± 0.02
REV	0.3 ± 0.01	54.8 ± 7.2	44.8 ± 1.0	3.11 ± 0.05

Table 3: Microbial diversity for the five boreal forest sites, based on standardised samples; *H* = Shannon diversity.

		Bacterial diversity		Fungal diversity	
Site	TSF (yrs)	No. OTUs	*H*	No. OTUs	*H*
RUS	14	219	4.4	479	3.7
LAD	135	258	4.6	402	4.0
GUO	182	190	3.8	399	4.3
TJA	255	211	4.2	405	3.0
REV	366	169	3.6	418	3.5

Table 4: Proportion of bacterial reads in each sample by taxonomic group; u/c = unclassified.

			RUS	LAD	GUO	TJA	REV
Acidobacteria	Acidobacteria	Acidobacteriales					
		Acidobacteriaceae					
		Edaphobacter sp.	42.8	35.8	8.6	17.1	12.7
		Other Acidobacteriaceae	4.3	6.9	3.0	6.0	5.3
		u/c Acidobacteriaceae	0.2	0.6	0.2	0.9	0.2
Actinobacteria	Actinobacteria	Actinomycetales					
		Frankineae	2.3	1.6	2.0	0.9	0.5
		Micrococcineae	0.4	1.9	1.4	3.1	0.4
		Other Actinomycetales	1.5	2.1	0.5	0.3	0.2
		u/c Actinomycetales	1.0	0.7	1.1	0.7	0.4
		Other Actinobacteria	0.1	1.6	0.0	0.8	0.2
		u/c Actinobacteria	0.1	0.0	0.0	0.1	0.1
Proteobacteria	α-proteobacteria	Caulobacterales	2.0	2.4	0.8	0.7	0.3
		Rhizobiales	10.3	3.1	1.5	2.0	1.0
		Rhodospirillales	11.4	7.9	4.9	4.4	3.7
		Other Alphaproteobacteria	0.2	0.2	0.6	0.7	0.0
		u/c Alphaproteobacteria	0.7	0.1	0.1	0.5	0.0
	β-proteobacteria	Burkholderiales					
		Burkholderiaceae					
		Burkholderia sp.	0.2	17.5	5.6	15.8	55.1
		Other Burkholderiales	0.6	0.5	1.4	0.6	1.0
		u/c Burkholderiales	2.4	0.8	0.8	0.4	0.7
	γ-proteobacteria	Enterobacteriales					
		Enterobacteriaceae					
		Serratia sp.	0.0	0.2	10.3	17.9	0.6
		Other Enterobacteriaceae	0.0	0.2	0.0	0.2	0.7
		Pseudomonadales					
		Pseudomonadaceae					
		Pseudomonas sp.	0.0	4.1	46.6	19.0	14.1
		Other Pseudomonadaceae	0.0	0.0	0.2	0.0	0.1
		u/c Pseudomonadaceae	0.0	0.0	0.6	0.1	0.2
		Xanthomonadales					
		Sinobacteraceae	15.5	2.7	4.1	0.7	1.3
		Xanthomonadaceae	0.0	0.0	0.0	0.0	0.0
		Dyella sp.	0.0	3.8	3.3	2.6	0.2
		Other Xanthomonadales	0.0	1.9	0.7	1.0	0.3
		u/c Gammaproteobacteria	0.2	0.1	0.2	0.1	0.0
	Other Proteobact.		0.4	0.3	0.0	0.0	0.0
	u/c Proteobacteria		0.3	0.4	0.2	0.2	0.2
Other phyla			1.8	1.9	1.1	2.4	0.2
Unclassified			1.5	0.6	0.3	0.6	0.2

Table 5: Proportion of fungal reads in each sample by taxonomic group; u/c = unclassified; [1] primarily from the sub-phylum Pezizomycotina; [2] primarily from the sub-phylum Pucciniomycotina

			RUS	LAD	GUO	TJA	REV
Ascomycota	Dothideomycetes	Capnodiales	0.2	0.7	0.8	0.7	0.5
		Dothideodiales					
		Dothioraceae					
		Aureobasidium sp.	0.0	1.2	1.4	5.8	7.0
		Other Dothioraceae	0.0	0.0	0.5	0.0	0.1
		Other Dothideomycetes	0.3	0.3	0.3	0.2	0.1
	Leotiomycetes	Helotiales					
		Helotiaceae					
		Crocicreas sp.	19.6	0.1	3.3	1.1	0.2
		Other Helotiales	1.0	11.0	1.9	1.9	4.0
		Unclassified Helotiales	10.1	14.0	32.5	2.7	6.6
		Other Leotiomycetes	2.5	0.3	0.7	0.9	0.8
		u/c Leotiomycetes	0.4	7.3	2.4	2.5	13.6
	Sordariomycetes	Hypocreales					
		Hypocreaceae					
		Hypocrea avellanea	0.0	2.5	0.0	29.3	0.4
		Trichoderma sp.	0.0	0.3	0.0	0.7	1.2
		Other Hypocreales	0.0	0.0	0.0	0.1	0.0
		u/c Hypocreales	0.0	0.0	0.0	0.1	0.0
		Sordariales					
		Chaetomiaceae					
		Chaetomium sp.	2.0	0.0	0.0	0.0	0.0
		Other Sordariales	0.0	0.1	0.2	0.2	0.2
		Xylariales					
		Amphisphaeriaceae					
		Pestalotiopsis sp.	0.0	0.0	0.0	7.4	5.6
		u/c Xylariales	0.1	0.0	0.0	0.0	0.1
		Other Sordariomycetes	0.0	0.0	0.7	0.1	0.2
	Other Ascomycota		0.7	0.8	0.4	1.0	0.7
	u/c Ascomycota[1]		8.7	11.2	33.5	20.8	20.3
Basidiomycota	Agaricomycetes	Agaricales					
		Mycenaceae					
		Mycena sp.	4.2	0.0	0.0	0.0	0.0
		Other Agaricales	0.1	0.5	0.0	0.3	0.3
		u/c Agaricales	0.0	0.2	0.2	0.1	0.7
		Cantharellales					
		Clavulinaceae					
		Clavulina sp.	1.6	0.1	0.7	0.1	0.0
		Other Agaricomycetes[2]	1.2	23.6	2.3	11.8	6.8
		u/c Agaricomycetes	4.7	5.1	2.6	1.8	3.0
	Tremellomycetes	Cystofilobasidiales					

		Cystofilobasidiaceae					
		Cystofilobasidium sp.	0.0	3.7	0.8	0.2	5.1
		Filobasidiales					
		Filobasidiaceae					
		Cryptococcus sp.	0.0	2.0	0.2	0.2	2.6
	Microbotryomycetes	Sporidiobolales					
		Incertae sedis					
		Rhodotorula sp.	0.5	3.7	3.9	2.8	4.0
		u/c Microbotryomycetes	0.0	1.6	0.2	0.1	0.8
	Other B. mycota		0.0	4.6	0.3	0.8	1.3
	u/c Basidiomycota		0.3	1.2	1.0	0.5	2.9
Zygomycota	*Incertae sedis*	Mortierellales					
		Mortierellaceae					
		Mortierella sp.	0.1	2.9	0.6	5.2	10.0
Unclassified			41.8	0.9	8.4	0.5	1.0

Table 6: Fungal lifestyles, based on the number of sequence reads from taxa that could be assigned to genus level or lower ('Total at genus level').

Site code	Time since fire (yrs)	Total at genus level	No. saprotroph reads	Proportion saprotroph (%)	No. mycorrhizal reads	% mycorrhizal
RUS	14	3283	2701	82	442	13
LAD	135	2116	1474	70	116	5
GUO	182	1480	1091	74	95	6
TJA	255	5735	1710	30	134	2
REV	366	4138	3141	76	52	1

FIGURE LEGENDS

Fig. 1. Selected edaphic factors (humus layer) and moss/humus layer thickness plotted against TSF. Dotted lines indicate 95% confidence intervals.

Fig. 2. CCA of bacterial community structure. TSF = time since fire; pH = pH of the humus layer. The two constraining variables accounted for 62% of the variance in the community data.

Fig. 3. CCA of fungal community structure. TSF = time since fire; pH = pH of the humus layer. The two constraining variables accounted for 64% of the variance in the community data.

Fig. 4. The relative abundance of the major bacterial phyla, showing the general increase in Proteobacterial reads with TSF, and the accompanying decrease in Acidobacteria. TSF increases from left to right.

Fig. 1

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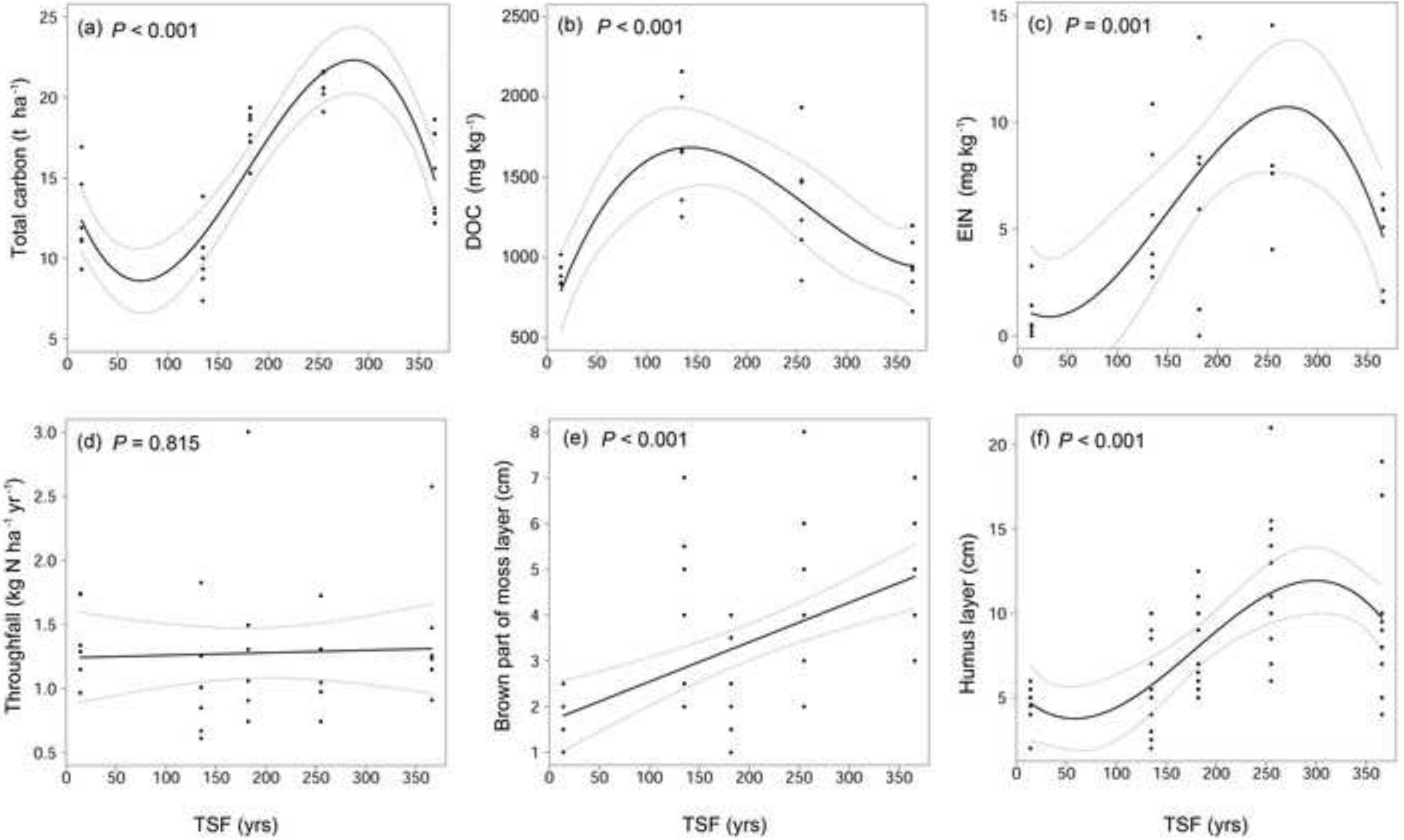

Fig, 2

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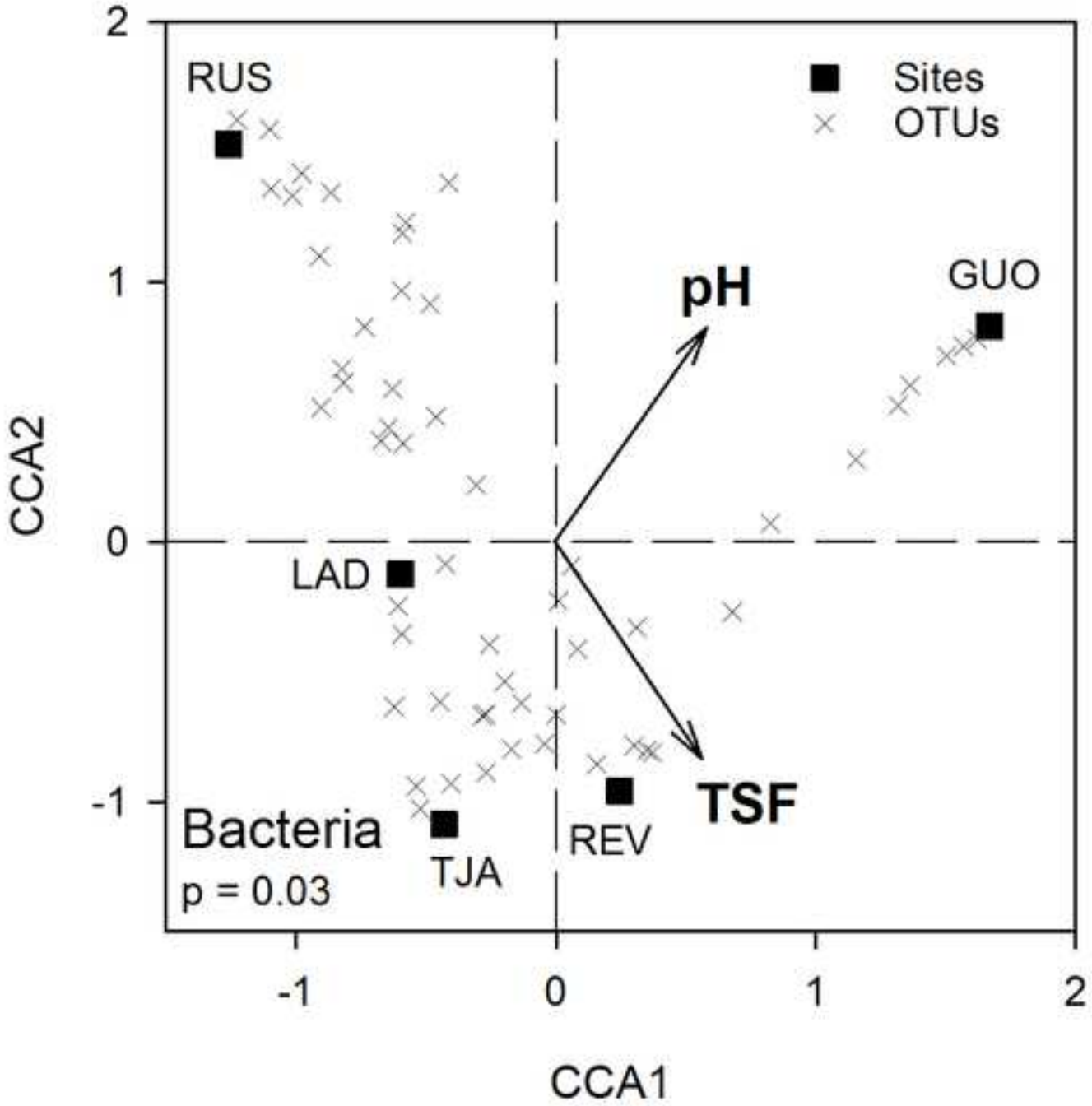

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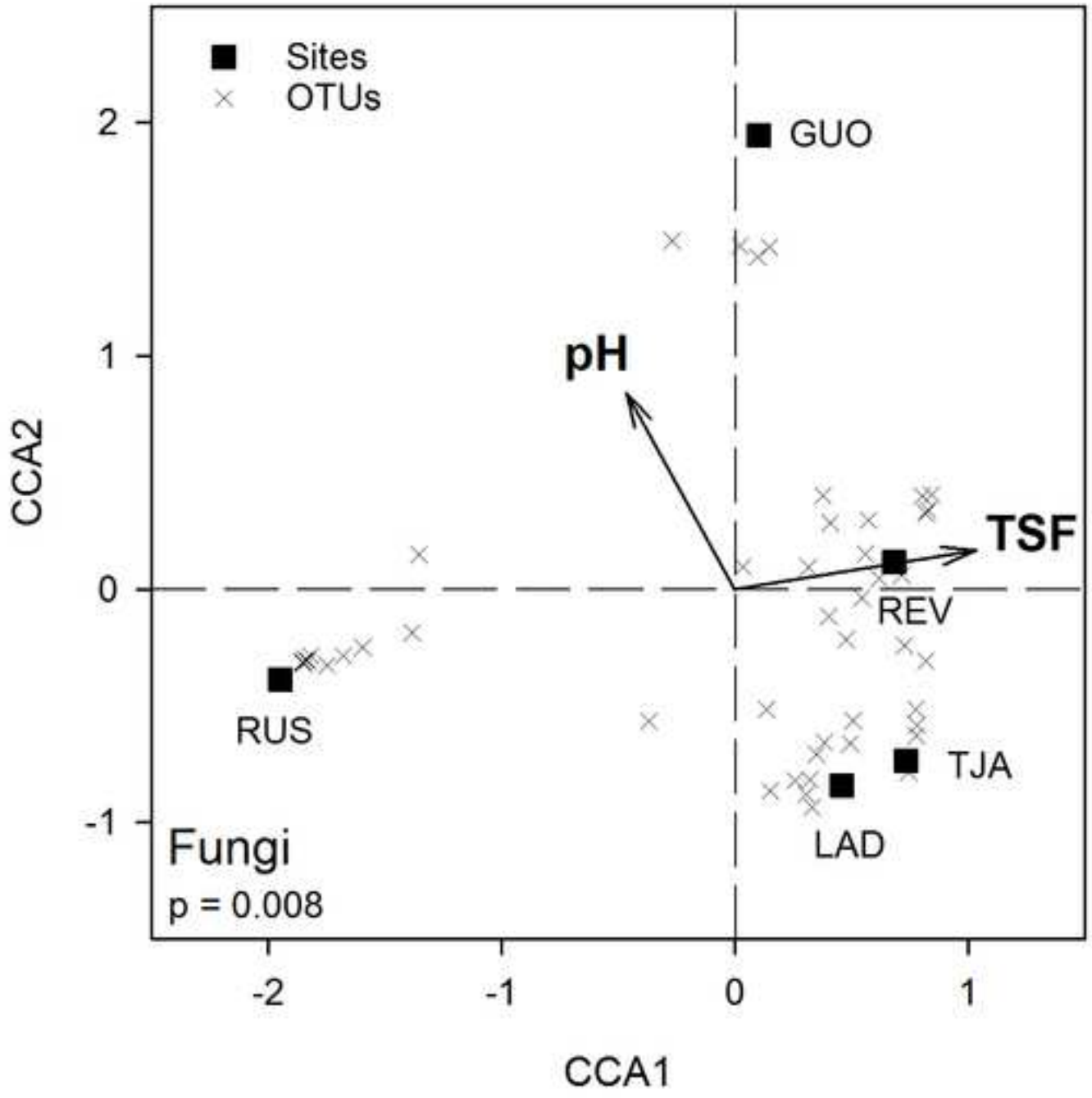

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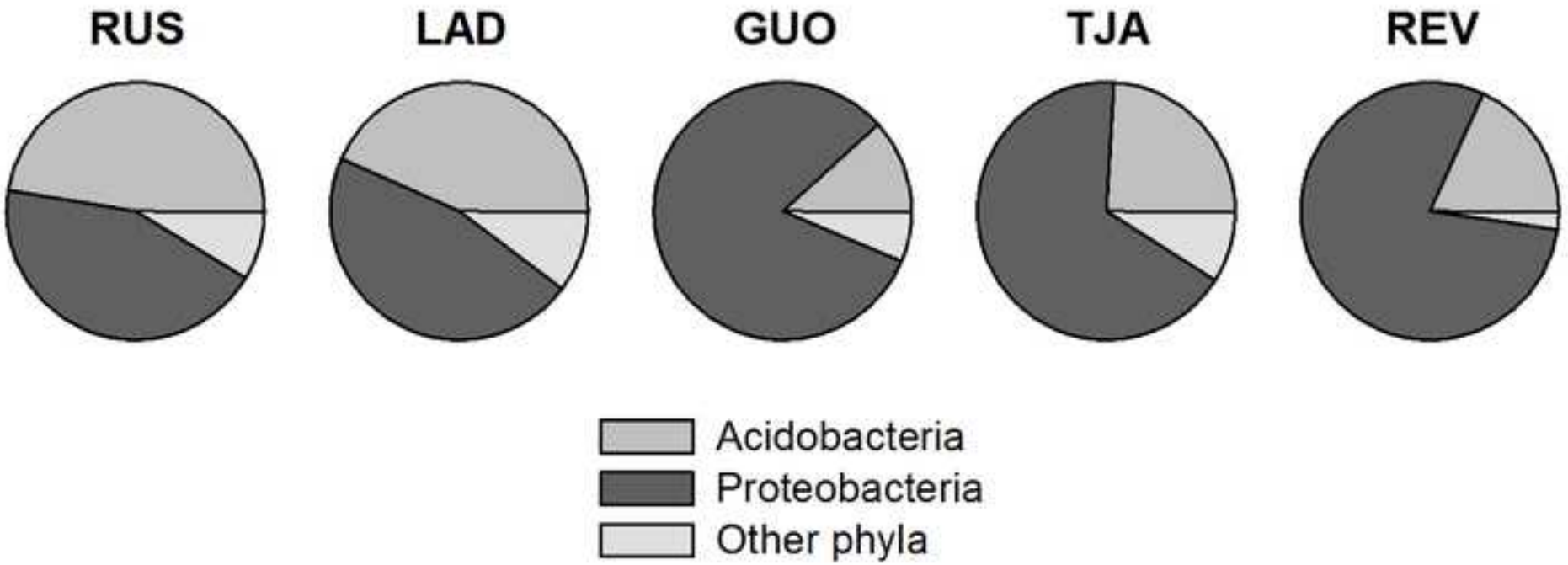

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